CLINICAL STUDY

Correlations of high-sensitivity C-reactive protein and atherosclerosis in Japanese type 2 diabetic patients

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Abstract

Background: The elevated level of high-sensitivity C-reactive protein (HSCRP) and aortic stiffness are associated with high mortality in type 2 diabetic patients. We tested the hypothesis that the HSCRP correlates with aortic stiffness and insulin resistance in type 2 diabetic patients.

Material and methods: The study consisted of 46 Japanese patients with type 2 diabetes and high HSCRP group (0.3–1.0 mg/dl, age: 57 ± 5 years, mean ± S.D.) and a control group of 55 age-matched patients with low HSCRP group (<0.3 mg/dl, 57 ± 6 years). Brachial–ankle pulse wave velocity (BaPWV) was measured by automatic oscillometric method and was used as an index of atherosclerosis.

Results: The body mass index (BMI) values (P <0.05) and waist circumferences (P <0.0005) and the waist-to-hip ratios (P <0.05) were higher in the high HSCRP group than in the low HSCRP group. The BaPWV was higher in the high HSCRP group than in the low HSCRP group (P <0.0001). Fasting plasma glucose (FPG; P <0.005) and insulin concentrations (P <0.0001), and the homeostasis model assessment (HOMA) index (P <0.0001), were higher in the high HSCRP group than in the low HSCRP group. Multiple regression analysis showed that HSCRP levels were independently predicted by BaPWV and HOMA index.

Conclusions: Our results indicate that the elevated level of HSCRP in Japanese patients with type 2 diabetes is characterized by increased aortic stiffness and insulin resistance, and that the BaPWV and HOMA index are independent predictors of HSCRP.

Introduction

It has been reported that measurement of inflammatory markers such as high-sensitivity C-reactive protein (HSCRP) is an important method for identifying individuals at risk for cardiovascular events (1, 2).

Pulse wave velocity (PWV) reflects arterial stiffness, and it has been demonstrated that carotid–femoral PWV relates to the severity of atherosclerosis (3) and predicts future atherosclerotic cardiovascular events (4). Recently, a simple method for measuring brachial–ankle PWV (BaPWV) has been reported (5–7). Moreover, BaPWV is a marker of severity of atherosclerosis (6, 7) and increased BaPWV is a risk factor for cardiovascular disease (7) and prognosis in patients with acute coronary syndrome (8).

Insulin resistance is linked to established risk factors for atherosclerosis such as hypertension, hyperlipidemia, and obesity, which subsequently accelerate the development and progression of atherosclerosis (9, 10).

Although plasma CRP is reported to be associated with insulin resistance in type 2 diabetic patients (11, 12), the significance of HSCRP in diabetic aortic stiffness has not been adequately investigated.

We hypothesized that increased levels of HSCRP are associated with BaPWV and insulin resistance in type 2 diabetic patients. To test our hypothesis, we compared BaPWV in addition to metabolic profiles in Japanese type 2 diabetic patients with low HSCRP levels and those with high HSCRP levels; independent predictors of the HSCRP in these populations were evaluated.

Subjects and methods

We screened 181 consecutive Japanese patients with type 2 diabetes mellitus who were admitted to our department in January 2006 and February 2007. Among these subjects, we enrolled 125 patients who did not have organic heart disease as determined by physical examination and routine laboratory tests, including serum electrolytes, serum creatinine, blood urea nitrogen, fasting plasma glucose (FPG), fasting immunoreactive insulin (F-IRI), chest X-ray, 12-lead
electrocardiography (ECG), echocardiography, treadmill exercise ECG, and thallium 201 cardiac scintigraphy.

All patients underwent a clinical examination to exclude the presence of secondary hypertension. Essential hypertension was defined as diastolic blood pressure ≥90 mmHg, systolic blood pressure ≥140 mmHg, or self-reported use of antihypertensive medication (13).

**Laboratory methods**

Blood was taken at 0700 h from the antecubital vein with the patient in the recumbent position after an overnight fast. All patients underwent routine laboratory tests including assays for serum electrolytes, serum total cholesterol, serum triglycerides, serum high-density lipoprotein, FPG, and F-IRI. Insulin resistance was evaluated by the homeostasis model assessment (HOMA) index: (fasting plasma insulin (μU/ml) × FPG (mmol/l))/22.5 (14). High-sensitivity assays for CRP were performed according to the previously described methods (Dade Behring, Tokyo, Japan) (15). By this assay, 46 patients were assigned to have high HSCRP (0.3–1.0 mg/dl; termed high HSCRP group) (16). We also recruited 55 age-matched patients who had low HSCRP (<0.3 mg/dl; low HSCRP group), who were selected from the original 125 enrolled patients. Patients who showed higher levels of CRP (i.e., >1.0 mg/dl) were excluded from the study (17). The clinical characteristics of patients in the low and high HSCRP groups are summarized in Table 1. In total, 31 of the 46 patients in the high HSCRP group and 35 of the 55 patients in the low HSCRP group met the criteria for essential hypertension and all of these patients were being treated with calcium channel antagonists, angiotensin-converting enzyme (ACE) inhibitors, and/or angiotensin II receptor blockers with diuretics. None of the patients were being treated with insulin. Dyslipidemia was defined as fasting triglycerides levels ≥200 mg/dl or a high-density lipoprotein-cholesterol (HDL-c) concentration <45 mg/dl for women and <35 mg/dl for men (13). Fifteen of the 46 patients in the high HSCRP group and 17 of the 55 patients in the low HSCRP group met the criteria for dyslipidemia. Patients treated with insulin were also excluded. Female patients who were pregnant or treated with any postmenopausal hormonal replacement or contraceptives were also excluded.

All subjects gave their written informed consent to participate in the study and the study protocol was approved by the ethics committee of the Oita Red Cross Hospital.

**Measurement of PWV**

Brachial–ankle PWV (BaPWV) was measured using a volume plethysmogram (Form/ABI, Colin Co., Komaki, Japan). The details of the measurement, validity, and reproducibility of this method have been reported previously (5–7). Briefly, the subject was examined while resting in the supine position with electrocardiogram electrodes placed on both wrists, a microphone for detecting heart sounds placed on the left edge of the sternum, and cuffs wrapped on both the brachia and ankles. The cuffs were connected to the plethysmogram sensor that determines the volume pulse form and the oscilometric pressure sensor that measures BP. The volume waveforms for the brachium and ankle were stored. The stored sample included sufficient waveform data. The characteristic points of waveforms were determined automatically according to the phase velocity theory. The components over 5 Hz were stored using a pass filter and the wave front was determined. The time interval between the wave front of the brachial waveform and that of the ankle waveform was defined as the time interval between the brachium and ankle (ΔTba). The distance between sampling points of BaPWV was calculated automatically according to the height of the subject. The path length from the suprasternal notch to the brachium (Lb) was obtained from superficial measurements and expressed using the following equation: 

\[ L_b = (0.2195 \times \text{height of the subject (cm)} - 2.0734) \]

The path length from the suprasternal notch to the ankle (La) was obtained from superficial measurements and expressed using the following equation: 

\[ L_a = (0.8129 \times \text{height of the subject (cm)} + 12.328) \]

Finally, the following equation was used to obtain BaPWV: 

\[ \text{BaPWV} = \left( (L_a - L_b) / \Delta T_{ba} \right) \]

All the studies, BaPWV was measured after at least 5-min rest. The interobserver coefficient of variation (CV) was 8.4% and the intraobserver CV was 10.0% (7).

**Anthropometric and body composition measurements**

The anthropometric and body composition characteristics of the patients were evaluated using the following parameters: height, body weight, BMI, waist circumference, hip circumference, and waist-to-hip ratio. BMI was calculated as weight/(height^2) (kg/m^2). The waist circumference was measured midway between the lower rib margin and the iliac crest and the hip circumference was measured at the widest circumference over the trochanter in standing subjects after normal expiration.

**Statistical analysis**

Data are presented as mean ± S.D. Differences between two groups were analyzed by the unpaired Student’s t-test, chi-square test, or Fisher’s exact probability test. A P value of <0.05 was considered statistically significant. Simple (Spearman’s rank) correlation coefficients between HSCRP and various parameters were calculated. Stepwise multiple regression analysis was then used to evaluate the association between the levels of HSCRP and other factors, such as the waist...
In our multivariate analysis, F4 were considered significant.

Results

As shown in Table 1, the mean ages of the high and low HSCRP groups were similar, and there were no significant differences between the groups with respect to gender, duration of diabetes, smoking habits, and administered medications. The BMI values, waist circumferences, and the waist-to-hip ratios were larger in the high HSCRP group than in the low HSCRP group (PZ0.0265, PZ0.0001, and PZ0.0409 respectively). The resting heart rate and systolic and diastolic blood pressures were not significantly different between the two groups. Regarding glucose metabolism, FPG and insulin concentrations and HOMA index values were higher in the HSCRP group than in the low HSCRP group (PZ0.0015, PZ0.0001 and PZ0.0001 respectively). However, there was no significant difference in hemoglobin A1c between the two groups. With regard to lipid metabolism, the concentration of serum triglyceride was higher and the concentration of serum HDL-cholesterol was lower in the high HSCRP group than in the low HSCRP group (PZ0.0002 and PZ0.0001 respectively), whereas serum total cholesterol levels were not significantly different between the groups. The concentration of uric acid was higher in the high HSCRP group than in the low HSCRP group (PZ0.0107). Parameters measuring renal function, the serum creatinine concentration was not significantly different between the groups.

Figure 1 shows the BaPWV in the low HSCRP group and in the high HSCRP group of type 2 diabetic patients. The BaPWV was higher in the high HSCRP group than in the low HSCRP group (2021 ± 256 cm/s vs 1493 ± 188 cm/s, PZ0.0001).

Table 2 depicts the correlation between the HSCRP level and age, BMI, and other variables in both the high- and the low HSCRP group. HSCRP levels were positively correlated with the BMI values, waist circumference, waist-to-hip ratio, triglyceride levels, FPG, fasting plasma insulin concentration, uric acid levels, HOMA index values, and BaPWV, and were negatively correlated with HDL-c levels. The concentration of uric acid was higher in the high HSCRP group than in the low HSCRP group (P=0.0107). Parameters measuring renal function, the serum creatinine concentration was not significantly different between the groups.

Table 3 depicts the correlation between the HSCRP level and age, BMI, and other variables in both the high- and the low HSCRP group. HSCRP levels were positively correlated with the BMI values, waist circumference, waist-to-hip ratio, triglyceride levels, FPG, fasting plasma insulin concentration, uric acid levels, HOMA index values, and BaPWV, and were negatively correlated with HDL-c levels.

Multiple regression analysis was performed using the stepwise procedure. The level of HSCRP was independently predicted by BaPWV and HOMA index (Table 3).
Discussion

In the present study, type 2 diabetic patients with HSCRP manifested increased arterial stiffness was evaluated by BaPWV. Among the metabolic parameters, fasting plasma concentrations of glucose and insulin and the HOMA index were higher in patients with high HSCRP than in those with low HSCRP. In addition, multiple regression analysis revealed that the levels of HSCRP in the patients could be independently predicted by the HOMA index values and BaPWV in Japanese patients with type 2 diabetes.

Recent studies have demonstrated a close relationship between elevated CRP and insulin resistance (18, 19). Yudkin et al. (18) reported that low but relatively elevated CRP in healthy subjects is related to insulin resistance when assessed by BMI, HOMA index, blood pressure, HDL-cholesterol, and triglyceride, and that increased proinflammatory cytokines, interleukin-6 and tumor necrosis factor-α (TNF-α), play an important role in the low level of chronic inflammatory state. Subsequently, by analyzing the non-diabetic population of the Insulin Resistance Atherosclerosis Study (IRAS) (20), Festa et al. (19) also reported that the level of CRP correlated with BMI, insulin sensitivity (assessed by i.v. glucose tolerance test), and fasting plasma levels of insulin and proinsulin. They suggested that CRP is not only a predictor of cardiovascular events but also an independent predictor of insulin sensitivity. In the present study, consistently, the level of HSCRP correlated with BMI, HDL-cholesterol, fasting plasma insulin concentration, and HOMA index. Being different from those two prior studies (18,19), our study enrolled type 2 diabetic patients who did not receive insulin treatment.

There are several reports indicating the relationship between levels of HSCRP and BaPWV (21, 22). Tomiyama et al. (21) reported the association between BaPWV and HSCRP in healthy Japanese men. Kim et al. (22) reported that the level of HSCRP is significantly associated with BaPWV in non-diabetic hypertensive patients. These reported subjects who underwent annual physical checkups using the same instrument as ours. This finding is in agreement with others (21, 22). The present study had some advantages and we examined abnormalities in glucose and insulin metabolism as well as lipid metabolism.

However, from the previous studies, the relationship between HSCRP, BaPWV, and insulin resistance has not been fully elucidated.

Although the specific mechanism that links the HSCRP level and BaPWV remains unknown, several mechanisms could explain our observations. First, CRP acts on vascular smooth muscle cells by upregulating the angiotensin type I receptor (23) and stimulating the migration and proliferation of smooth muscle cells, in addition to the production of reactive oxygen species.

Table 2 Correlations between high-sensitivity C-reactive protein and various parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.067</td>
<td>0.5046</td>
</tr>
<tr>
<td>Duration of diabetes mellitus</td>
<td>0.082</td>
<td>0.4160</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.246</td>
<td>0.0131</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.276</td>
<td>0.0052</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>0.022</td>
<td>0.8270</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.204</td>
<td>0.0409</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.160</td>
<td>0.1096</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.130</td>
<td>0.1941</td>
</tr>
<tr>
<td>Heart rate</td>
<td>0.161</td>
<td>0.1073</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.098</td>
<td>0.3321</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.211</td>
<td>0.0339</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol</td>
<td>0.328</td>
<td>0.0008</td>
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<tr>
<td>Uric acid</td>
<td>0.224</td>
<td>0.0243</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.260</td>
<td>0.0087</td>
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<tr>
<td>Fasting immunoreactive insulin</td>
<td>0.519</td>
<td>0.0001</td>
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<tr>
<td>Homeostasis model assessment index</td>
<td>0.560</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hemoglobin A1c</td>
<td>0.122</td>
<td>0.2239</td>
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<tr>
<td>Creatinine</td>
<td>0.092</td>
<td>0.3591</td>
</tr>
<tr>
<td>Brachial–ankle pulse wave velocity</td>
<td>0.536</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 3 Stepwise regression analyses between high-sensitivity C-reactive protein (HSCRP) and various parameters.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>Standard regression coefficient</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>To HSCRP intercept</td>
<td>−0.524</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaPWV</td>
<td>0.313</td>
<td>0.810</td>
<td>0.344</td>
<td>6.902</td>
</tr>
<tr>
<td>HOMA index</td>
<td>0.127</td>
<td>0.029</td>
<td>0.394</td>
<td>10.546</td>
</tr>
</tbody>
</table>

BaPWV, brachial–ankle pulse wave velocity; HOMA, homeostasis model assessment.
Inhibition of rennin–angiotensin system (RAS) by angiotensin receptor blocker (ARB) or ACE inhibitor (ACEI) resulted in a reduction of vascular smooth muscle cell proliferation (24, 25), and improved the BaPWV which is a parameter of atherosclerosis (26). In addition, angiotensin II, via the type-1 (AT1) receptor, stimulates NADPH oxidase and enhances production of reactive oxygen species (ROS) (27), which in turn contributes to endothelial dysfunction by inactivating nitric oxide (NO) (28).

Secondly, CRP has a direct effect on the endothelial cells and induces the secretion of specific chemokines, particularly monocyte chemokine protein-1, adhesion molecules, and E-selection (29), whereas it decreases the expression of NO synthase (30).

Furthermore, previous study demonstrated that several chemokines accelerated atherosclerosis (31), while the inhibition of chemokines reduced the atherosclerosis (32). In addition, activation or inhibition NO differentially regulates atherosclerosis (33).

In contrast to these reports, a recent study showed that pure human CRP increases rather than decreases, the bioavailability of NO in blood vessels in vitro (34).

ROS, produced by CRP through RAS activation, interacts with NO and forms nitrogen oxide, a strong toxic agent to endothelium (35, 36).

Furthermore, Steinberg et al. (37) reported that insulin-resistant states such as diabetes and obesity are associated with decreased endothelium-dependent vasodilation (37), and arterial compliance may be a partially NO-dependent process (38). In addition, insulin has been shown to induce vascular smooth muscle proliferation and migration in cell cultures (39).

Animal studies have also suggested that, after balloon endothelial injury, hyperinsulinemia induces an increase in neointimal hyperplasia that was not seen in rats with streptozotocin-induced diabetes without hyperinsulinemia (39).

The novel and important findings of the present study is that type 2 diabetic patients with elevated HSCRP levels had increased aortic stiffness and insulin resistance compared with those with low HSCRP.

These results suggest that aortic stiffness directly or indirectly relates to the insulin resistance. In addition, these factors are independent for each.

There are several limitations to this study. First, 67 and 64% of our patients with high HSCRP and low HSCRP respectively had been diagnosed earlier with associated essential hypertension. All these patients were being treated with one or more antihypertensive drugs, including ACE inhibitors, angiotensin II receptor blockers, and calcium channel antagonists, prior to enrollment. In this regard, all of these three drug classes have been reported to improve insulin resistance (40, 41) and aortic stiffness (42, 43). In addition, 33 and 31% of our patients with high HSCRP and low HSCRP respectively had been diagnosed with dyslipidemia. All these patients were being treated with statin drugs, including simvastatin and pravastatin prior to enrollment. In this regard, all of these drug classes have been reported to decrease the level of CRP (44, 45).

Therefore, these medications might have beneficially affected our results. As for anti-diabetic medications, a considerable number of patients were being treated with sulfonylureas and/or α-glucosidase inhibitors, while only one patient in each group was treated with pioglitazone, an insulin-sensitizing drug reported to reduce HSCRP in type 2 diabetic patients (46).

Secondly, no patients enrolled in the present study underwent coronary angiography. Although ischemic heart disease could not be completely excluded, severe coronary artery disease was unlikely to be present in view of the normal treadmill exercise ECG testing and thallium-201 cardiac scintigraphy. Finally, it has been reported recently that there is an association between CRP gene single nucleotide polymorphisms (SNP) and both the serum CRP level and BaPWV (47). We could not measure SNP in the present study. Further clinical studies to examine the relationship between BaPWV and insulin resistance with CRP gene polymorphisms are needed.

In conclusion, our findings suggest that higher levels of HSCRP are associated with aortic stiffness and insulin resistance, and that BaPWV and the HOMA index are independent predictors of HSCRP in type 2 diabetic patients.

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Received 12 June 2007
Accepted 26 June 2007